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Formulation And Test Of Antioxidants And Effectiveness Of Toner Preparations From Pumpkin Fruit Extract (Cucurbita Moschata Duch) Using The Dpph (2,2 Diphenyl - 1-Picrylhydrazyl) Method

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Abstract

Pumpkin (Cucurbita moschata Duch.) is rich in natural antioxidants to overcome skin xerosis which has a high prevalence in Indonesia, but stable toner formulations are still limited. This study aims to develop a pumpkin extract toner and test its antioxidant activity. This type of quantitative experimental research with variations in extract concentration (0%, 5%, 10%, 20%). The population of fresh fruit from Karanganyar, simplicia samples and 9 female panelists aged 20-25 years. Instruments include a rotary evaporator, pH meter, skin moisture meter, UV-Vis spectrophotometer; SPSS analysis (One-Way ANOVA) and IC50 DPPH regression. The results showed that all formulas met physical standards (pH 4.6-5.6, homogeneous, non-irritant, moisture 46-55%), with very strong IC50 (F0: 44.02 µg/mL; F1: 40.19; F2: 39.65; F3: 38.86 µg/mL), a positive dose-response trend. Formula F3 is optimal as a natural antioxidant toner.

Keywords: Antenatal Care, Knowledge, Public Health Students, Third Trimester, Maternal Health.

INTRODUCTION

Indonesia is rich in medicinal plants, such as pumpkin (Cucurbita moschata Duch), which is used as a food source, medicine, and natural cosmetic ingredient due to its antioxidant content. Pumpkin fruit contains carotenoids, tocopherols, phenolics, flavonoids, polysaccharides, and minerals that contribute to its strong antioxidant activity with an IC50 of 30.75 ppm using the DPPH method, making it highly potential for fighting free radicals.

Pumpkin is beneficial for skin care due to its anti-inflammatory and antioxidant properties, which can help treat acne, premature aging caused by pollution and UV rays, and dry skin (xerosis). The prevalence of xerosis in adults is approximately 30% and increases with age, as the stratum corneum loses moisture.

Although pumpkin is widely known as a food ingredient, its use as a cosmetic ingredient is still limited, including in toner preparations that function to enhance cleansing, remove residue, and maintain skin softness with astringents and humectants. Research shows that pumpkin extract has cytoprotective, anti-inflammatory, and photoprotective effects on human skin cells. However, toner formulations based on this extract have not been widely explored to optimize its antioxidant activity in cosmetics.

The main problem is the lack of stable toner formulations from pumpkin extract that meet physical standards and maintain antioxidant potential to treat xerosis and skin aging. Furthermore, IC50 data on toner preparations is still limited, even though it is necessary to confirm their antioxidant strength. This study tested whether pumpkin extract is an active antioxidant, can be formulated as a good toner, and has a competitive IC50.

This study aims to evaluate the antioxidant potential of pumpkin extract, the best toner formulation that meets physical quality standards, and the IC50 of the final product. The urgency lies in the need for natural alternatives to address the high prevalence of dry skin in Indonesia, reducing dependence on synthetic cosmetics. The novelty is the development of a pumpkin toner with comprehensive antioxidant testing, complementing previous limited studies.

RESEARCH METHODS

Research Types and Methods

This study uses a quantitative experimental approach to test the effect of varying concentrations of pumpkin (Cucurbita moschata Duch.) fruit extract on the physical characteristics and antioxidant activity of toner preparations. The experimental method involves manipulating independent variables in the form of extract concentrations (0%, 5%, 10%, 20%) under controlled conditions to observe their effects on dependent variables such as the IC50 value of the DPPH method and physical parameters such as pH and skin moisture [Sugiyono, 2021][Sudaryono, 2022]. This approach ensures a clear causal relationship through structured treatments, including preparation of simplex, 96% ethanol maceration extraction, toner formulation, characteristic testing, and antioxidant analysis [Emzir, 2021]

The research was conducted at the Microbiology Laboratory of the Undergraduate Pharmacy Study Program, Faculty of Health Sciences, Duta Bangsa University, Surakarta, for 4 months (March-June 2025), with plant determination at UPF Hortus Medicus RSUP Dr. Sardjito Tawangmangu and ethical clearance at Muhammadiyah University of Purwokerto [Creswell & Creswell, 2023] The independent variables include extract concentration, the dependent variable is antioxidant activity (IC50 DPPH), and the control variables include pH (4.2-6.5), homogeneity, skin irritation, and humidity [Rafika, 2021].

Data Analysis Tools and Techniques

The main instruments include a blender, analytical balance, rotary evaporator, pH meter, UV-Vis spectrophotometer, skin moisture meter, as well as materials such as pumpkin fruit, 96% ethanol, DPPH, glycerin, Tween 80, nipagin, nipasol, propylene glycol, and distilled water [Hutahaein, 2023][Zahro, 2023]. Measurement techniques include organoleptic tests, water content (moisture analyzer <10%), drying loss, ash content, phytochemical screening (saponins, flavonoids, alkaloids), as well as preparation characteristics such as homogeneity, irritation (10 female volunteers aged 20-25 years), and skin moisture [Rochayati et al., 2024].

Data analysis using SPSS for normality test, followed by One-Way ANOVA if normal or Kruskal-Wallis if not, to compare the significance between toner formulas (F0-F3). [Hutahaein, 2023][Sugiyono, 2021] The percentage of DPPH inhibition was calculated using the formula %Inhibition = [(Abs blank - Abs sample)/Abs blank] × 100, plotted linear regression for IC50, ensuring statistical validity [Sudaryono, 2022][Lismawati et al., 2021].

Population and Sample

The study population was fresh pumpkin (Cucurbita moschata Duch.) from Peiring Village, Mojogedang District, Karanganyar Regency, while the sample was a representative simplicia and extract for toner formulation [Hutahaein, 2023][Emzir, 2021] The sample was processed into 60 mesh powder, extracted by maceration (1:4 ethanol 96%, 5 days), producing a thick ethanol-free extract for varying concentrations. [Sari et al., 2023]. Volunteers for the irritation and moisture test consisted of 10 women aged 20-25 years, selected purposively to represent the normal skin population [Khaira et al., 2022][Creswell & Creswell, 2023].

Research Procedures

The procedure begins with the collection of simplicia: wash, cut, dry (cover with black cloth), grind with a blender, filter through 60 mesh, standardize (drying shrinkage, water content <10%, ash), then macerate 500 g of simplicia (1:4 ethanol 96%, 5 days + re-maceration 2 days), evaporation 50°C [Hutahaein, 2023][Saerang et al., 2023] The extract was standardized organoleptically, water content,

ethanol-free, phytochemical screening, followed by F0-F3 toner formulation (0-20% extract, 0.18% nipagin, 0.02% nipasol, 10% glycerin, 10% propylene glycol, Oleum Rosae qs, Tween 80 0.5%, aquades ad 100) [Zahro, 2023]. Toner making: dissolve extract-aquades, add humectant, Oleum Rosae-Tween 80 slowly, stir magnetic stirrer 10 minutes (1000 rpm) [Halimatushadyah et al., 2024] Characteristic testing: organoleptic, homogeneity (object glass), pH, irritation, moisture (skin moisture meter pre-post 2 hours). DPPH test: stock solution 100 ppm, maximum λ (400-800 nm), operating time 30 minutes, concentration series 20-100 ppm for extract/toner/vitamin C, calculate inhibition and IC50 regression [Asjur et al., 2023][Diyana Wulandari & Gigih Kenanga Sari, 2024].

RESULTS AND DISCUSSION

Plant Determination

The results of the determination of yellow pumpkin fruit (Cucurbita moschata Duch.) from the Cucurbitaceae family were carried out at the UPF Health Services of Dr. Sardjito General Hospital, Tlogodringo Aromatic Garden, Tawangmangu, Karanganyar, Central Java, confirming the correctness of the identity of the plant for research.

Sample Preparation

The plants used in this research are yellow pumpkins that are still fresh and without defects. Samples of yellow pumpkins (Cucurbita moschata Duch) were obtained from Deisa Peireing, Mojogeidang District, Karanganyar Regency.

Making Simple Drugs

Table 1 Calculation of Pumpkin Fruit Yield

Wet weight (g)	Dry Weight(g)	Yield(%)
5,000	500	10%

The collection of pumpkin (Cucurbita moschata Duch.) fruit peel simplicia yielded a wet weight of 5,000 grams, which after drying became 500 grams with a yield of 10%. The powder was ground using a grinder and sieved with mesh no. 60 to reduce particle size and increase surface area, thus optimizing the extraction of active substances [Handoyo & Pranoto, 2020].

Standardization of Simple Drugs

1. Organoleptic Test

Table1Organoleptic test results of simple powder

Color	Aroma	Form	Flavor
Yellow	Typical yellow pumpkin fruit	Seefine powder	Typical yellow pumpkin fruit

2. Drying Loss

Table 3 sults of drying shrinkage of simple drugs

Replication	Crucible Weight + Sample Before Heating(g)	Crucible + sample weight after heating (g)	Drying loss (%)
1	48.4	45.3	6.4%
2	49.4	45.1	8.7%
3	48.2	44.1	8.5%
	Average		7.8%

The results of the drying shrinkage test of pumpkin fruit (Cucurbita moschata Duch.) simplicia with three replications showed consecutive values of 6.4%, 8.7%, and 8.5%, all of which met the Indonesian Herbal Pharmacopoeia (2017) standards because they did not exceed 10%. This parameter is non-specific to evaluate the amount of compounds lost during drying, thus ensuring the quality of the simplicia [Loveina & Hari, 2023].

3. Water Content Test

Table 4. Results of water content test of simple drugs

Replication	Sample weight	Results	Condition
1	2	6.04	Less than 10%
2	2	6.07	Less than 10%
3	2	7.83	Less than 10%

The water content test of pumpkin (Cucurbita moschata Duch.) fruit simplicia using a moisture analyzer with three replications on an initial sample of 2 g produced values of 6.04%, 6.07%, and 7.83%, respectively, which met the quality standards (<10%). High water content can damage the simplicia by triggering microbial growth and fragility, so this test determines the maximum limit of compound loss during drying [Karimah, 2020][Rochayati et al., 2024].

4. Ash Content Test

Table 5. Ash content test results

Weight of simple substance (g)	Empty crucible weight (g)	Weight of ash + crucible (g) after heating	Results
2	27.83	46.13	9.15%

The total ash content test results for pumpkin (Cucurbita moschata Duch.) fruit simplicia of 9.15% meet the Indonesian Herbal Pharmacopoeia standards which require a value of no more than 10% [Yana et al., 2022]. This parameter indicates a low content of inorganic impurities, thus ensuring the purity of the simplicia for the next extraction stage.

Extract Preparation

Table 6. Results of Pumpkin Extraction

Weight of simple substance (g)	Extract weight (g)	Yield (%)
500	70.51	14,102

Maceration extraction of pumpkin fruit (Cucurbita moschata Duch.) produced a yield of 14.102%, which reflects the efficiency of the process in extracting active substances from 500 g of simplex. [Hutahaein, 2023] Stirring during maceration ensures even distribution of 96% ethanol solvent to bind polar components, while optimal temperatures below 60°C prevent degradation of bioactive compounds such as phenolics that are susceptible to oxidation [Zumaro et al., 2021][Saerang et al., 2023].

Extract Standardization

1. Organoleptic Test

Table 7. Results of organoleptic test of extract

Color	Aroma	Flavor	Form
Yellow peKat Keblackish	Typical yellow pumpkin fruit	Typical yellow pumpkin fruit	Tontal pecat

2. Water Content Test

Table 8. Results of the water content test of the extract

Initial Weight	Results	Condition
2	8.53%	Less than 10%

The water content test of pumpkin (Cucurbita moschata Duch.) fruit extract yielded a value of 8.53%, which meets the quality standards for thick extracts (<10%). This low water content prevents microbial growth and maintains the stability of active compounds during storage [Sambodeh et al., 2022][Sari et al., 2023].

3. Ethanol Free Test

Table 9. Ethanol-free test results

Sample	Results
Eextract epumpkin fruit tanol	There is no smell esteretanol (-)

The results of the ethanol-free test on pumpkin fruit extract (Cucurbita moschata Duch.) showed no typical alcohol ester odor, confirming the purity of the extract without ethanol residue contamination [Sari et al., 2023] This test is important to ensure the safety and stability of the extract before toner formulation [Hutahaein, 2023].

Phytochemical Screening

Table 10. Phytochemical Screening Results of Pumpkin Fruit Extract

Compound	Procedure	Test Results	Note
Flavonoid	Seelots of 2ml eextract added 2ml of hot water and taught.he continued, adding 0.1 grams of semagnesium powdersium 1ml HCL mixture shaken.Epositive extract mecontains flavonoids whenrbefor my colorred, yellow or orange	Beorange color	+

Alkaloid	Seemany 10 teteseextract is inserted intoin the re tubeaction plus 2 tetes HCl 2N,kethen divided intoin bebere tube rapaaction. Seeach tube is added dewith each personreMaye's actionr terbefor ewhite or yellow sediment and peadditional peredragee actionDroff terbefor eorange sediment.	Terbefor esediment (PereMaye's actionr) Terbefor eorange sediment (peredragee action(ndroff)	+
Saponin	Seelots of 2ml eextract and 5 teates water is put intoin the re tubeaction tothen shake itjust as strong as10 years oldtick, Epositive extract mecontains saponin when terbefor foam seheight 1-10cm seless than 10 minutesnit and on peadditional 2 tetes HCl 2N foam testeady tap.	Stable Foam	+

Phytochemical screening of ethanol extract of pumpkin fruit (Cucurbita moschata Duch.) showed positive results for flavonoids, alkaloids, and saponins, consistent with previous studies [Diyana Wulandari et al., 2024][Wardhani & Pardeidei, 2022]

Flavonoid test with magnesium and HCl produces an orange color due to the complexation of the flavonoid phenolic hydroxyl group with Mg^{2+} ions under acidic conditions, forming a stable C6-C3-C6 structure [Wardhani & Pardeidei, 2022] A positive alkaloid test is characterized by a yellow precipitate (Dragendorff Mayer reagent) and orange (Dragendorff reagent), confirming the presence of heterocyclic nitrogen compounds [Diyana Wulandari et al., 2024]

The saponin test produced stable foam (1-3 cm, >10 minutes) that was resistant to the addition of 2N HCl due to the natural surfactant properties of saponins which reduce the surface tension of water [Wardhani & Pardeidei, 2022] These three compounds contribute to the antioxidant and cosmetic potential of the extract for toner preparations.

Making Face Toner from Pumpkin Fruit Extract

Table 11. Formulation of Face Toner Preparation with Pumpkin Fruit Extract

Material	**Concentration %**				**Information**
	F0	F1	F2	F3	
Epumpkin fruit extract Yellow	0	5	10	20	Active ingredients
Nipagin	0.18	0.18	0.18	0.18	Pemessing aroundt
Nipasol	0.02	0.02	0.02	0.02	Pemessing aroundt
GliseRin	10	10	10	10	Humewe
Propileglycol	10	10	10	10	Humewe

OleUm Rosae	qs	qs	qs	qs	Pefragrant
Tween 80	0.5	0.5	0.5	0.5	Surfactant
Aquadest	Add 100	Add 100	Add 100	Add 100	Pelate

Antioxidant face toner is made from pumpkin (Cucurbita moschata Duch.) fruit extract with four formulas: F0 (0%), F1 (5%), F2 (10%), and F3 (20%). The concentration variations aim to evaluate differences in physical quality (pH, homogeneity, stability) and antioxidant activity of the preparations [Zahro, 2023].

Toner Preparation Evaluation

1. Organoleptic Test

Table 12. Results of organoleptic test of face toner

Preparation	Color	Texture	Aroma
F0	BeNing	Tontal rather liquid	Oleum rosae
F1	White tobrass	Tontal rather liquid	Oleum rosae
F2	Yellow	Tontal rather liquid	Oleum rosae
F3	Yellow Pecat	Tontal rather liquid	Oleum rosae

Organoleptic tests showed that formula F0 was clear, F1 (5%) was clear white, F2 (10%) was yellow, and F3 (20%) was dark yellow, according to the influence of the concentration of pumpkin fruit extract which is rich in carotenoids [Zahro, 2023] All formulas had a thick, slightly runny texture and a consistent Oleium Rosae aroma, indicating the stability of the toner preparation.

2. pH test

Table 13. Face Toner pH test results

Preparation	Replication 1	Replication 2	Replication 3	Average
F0	4.4	4.6	4.8	4.6
F1	4.9	4.8	4.9	4.8
F2	5.0	4.9	5.1	5
F3	5.6	5.6	5.6	5.6

The pH test on four face toner formulas with pumpkin (Cucurbita moschata Duch.) extract produced an average of 4.6-5.6, which is within the safe range of 4.5-6.5 to prevent skin irritation. [Ardini, 2021] This value corresponds to normal skin pH (4.7-5.75) which supports the acid mantle as a natural protector. The One-Way ANOVA results showed a significance of 0.000 ($p \leq 0.05$), indicating a significant difference between formulas due to variations in extract concentration [Zahro, 2023] This pH variation does not interfere with the overall safety of the preparation [Pratasik et al., 2019].

3. Homogeneity Test

Table 14. Results of the Homogeneity Test of Face Toner Preparations

Preparation	Results	Information
F0	Homogeneousn meaverage not tesee there are particlesI besar	Good
F1	Homogeneousn meaverage not tesee there are particlesI besar	Good
F2	Homogeneousn meaverage not tesee there are particlesI besar	Good
F3	Homogeneousn meaverage not tesee there are particlesI besar	Good

The homogeneity test results for the four face toner formulas containing pumpkin (Cucurbita moschata Duch.) extract showed a uniform distribution of ingredients without any grains or coarse particles when visually observed [Fitria & Padua Ratu et al., 2022]. This criterion meets the standards for cosmetic preparations that require perfect mixing of Tween 80 as a surfactant and distilled water as a solvent [Zahro, 2023]. Consistent homogeneity between F0-F3 indicates the stability of the formulation regardless of variations in extract concentration.

4. Irritation Test

Table 15. Results of Irritation Test of Face Toner Preparations

Formula	Reaction	Information
F0	Redness, Itching, Swelling	No irritation occurs
F1	Redness, Itching, Swelling	No irritation occurs
F2	Redness, Itching, Swelling	No irritation occurs
F3	Redness, Itching, Swelling	No irritation occurs

An irritation test on 9 panelists by applying a face toner with pumpkin (Cucurbita moschata Duch.) extract to the ear area for 15 minutes showed no signs of irritation such as redness, itching, or swelling [Hutahaein, 2023]. These results confirm the safety of all formulas (F0-F3) for topical use, according to cosmetic patch test criteria that require visual observation without negative reactions. Natural extract preparations like this are generally safe due to their mild phytochemical content.

5. Humidity Test

Table 16. Humidity test results

Preparation	Replication 1	Replication 2	Replication 3	Average
F0	54.2	54.4	54.8	54.4
F1	55.3	55.5	55.4	55.4
F2	57.5	55.3	54.8	55.7
F3	55.5	56.2	54.8	55.5

The results of the skin moisture test with a skin moisture meter showed normal/moist values (46-55%) after applying a face toner containing pumpkin (Cucurbita moschata Duch.) extract [Hutahaein & Kisno Saputri, 2022]. This moisture is influenced by the increased skin water content thanks to glycerin (10%) as a humectant that binds and retains water from the environment to the stratum corneum [Hutahaein, 2023]. The use of this humectant ensures optimal hydration without changing the extreme moisture category.

Antioxidant Activity Testing Using the DPPH Method

1. Wavelength Measurement Results

The maximum wavelength was determined by mixing a DPPH solution with ethanol and measuring the wavelength using a UV-Vis spectrophotometer. The solution was incubated for 30 minutes in the dark. Measure the absorbance of the solution using a UV-Vis spectrophotometer at wavelengths of 400–800 nm and determine the maximum wavelength (Hidayati & Masykuroh, 2023).

According to the research findings, the maximum wavelength obtained was 530 nm, with an absorption value of 0.720.

2. Operating Time Results

The purpose of determining the operating time was to identify the optimal time by monitoring the stability of the absorbance values at 2-minute intervals over a 30-minute period. Stable absorbance values were observed at 14, 15, and 16 minutes, with an absorbance value of 0.678.

3. Antioxidant Test of Reference Solution

Table 17. Results of Vitamin C Antioxidant Activity

Concentration (ppm)	% Inhibition	IC50(µg/mL)
20	36.66	
40	39.44	
60	43.75	7.95
80	46.38	
100	51.25	

Vitamin C as a comparator in the DPPH method antioxidant test produced an IC50 value of 7.95 µg/mL, which is categorized as very strong because it is <50 µg/mL [Cahaya, 2020]. The smaller the IC50 value, the higher the compound's ability to inhibit 50% of DPPH free radicals, making vitamin C an effective reference standard for comparing the potential of pumpkin extract.

4. Antioxidant Test of Yellow Pumpkin Fruit Extract Sample Solution

Table 18. Results of the Extract Antioxidant Test

Concentration (ppm)	% Inhibition	IC50(µg/mL)
20	29.86	
40	40.97	
60	51.8	42.37
80	58.88	
100	64.72	

Pumpkin fruit extract (Cucurbita moschata Duch.) showed very strong antioxidant activity with an IC50 value of 42.37 µg/mL using the DPPH method, because it was below the threshold of <50 µg/mL [Cahaya, 2020]. The lower the IC50 value, the higher the compound's capacity to capture 50% of free radicals, which is supported by the flavonoid, phenolic, and carotenoid content in the extract [Wulandari et al., 2022]. These results are close to the standard vitamin C (7.95 µg/mL) as a strong comparison.

5. DPPH Activity Results of Facei Toneir of Yellow Pumpkin Fruit.

Table 19. Results of IC50 Values in Face Toner Preparations

Sample	Concentration (ppm)	Average Absorbance	% Inhibition	IC50 (ppm)
F0	20	0.502	30.27	44.02
	40	0.445	38.19	
	60	0.375	47.91	
	80	0.353	50.97	
	100	0.252	65	
F1	20	0.404	43.88	40.19
	40	0.375	47.91	
	60	0.305	57.63	
	80	0.253	64.86	
	100	0.234	67.5	
F2	20	0.504	30	39.65
	40	0.455	36.80	
	60	0.394	45.27	
	80	0.356	50.55	
	100	0.241	66.52	
F3	20	0.507	29.58	38.86
	40	0.445	38.19	
	60	0.378	47.5	
	80	0.316	56.11	
	100	0.255	64.58	

The DPPH test showed that formula F0 (0% extract) had an IC50 of 44.02 µg/mL, which originated from the residual activity of glycerin, propylene glycol, and Tween 80 through physical mechanisms such as hydrogen bonds and changes in media polarity, not bioactive redox reactions. [Hutahaein, 2023][Zahro, 2023] The active formulas F1 (5%: 40.19 µg/mL), F2 (10%: 39.65 µg/mL), and F3 (20%: 38.86 µg/mL) were all very potent (<50 µg/mL) thanks to flavonoids, phenolics, carotenoids, and tocopherols as electron/hydrogen donors. [Cahaya, 2020][Wulandari et al., 2022]

The higher the concentration of pumpkin (Cucurbita moschata Duch.) fruit extract, the lower the IC50 and the higher the inhibition percentage, indicating a positive dose-response relationship. Vitamin C as a positive control has an IC50 of 7.95 µg/mL (very strong), while the decrease in DPPH absorbance with solution concentration confirms effective radical scavenging activity in all samples. [Cahaya, 2020] Although the IC50 of F0-F3 is relatively close, the bioactive mechanism of the extract is pharmacologically superior to the physical-chemical effects of the basic formula.

CONCLUSION

This study successfully developed a face toner preparation based on pumpkin fruit extract (Cucurbita moschata Duch.) with a concentration variation of 0-20%, which met physical standards such as pH 4.6-5.6, perfect homogeneity, safe from irritation in 9 panelists, and increased skin moisture to the normal range (46-55%). The antioxidant activity of the DPPH method showed very strong results in all formulas (IC50 F0: 44.02 µg/mL; F1: 40.19 µg/mL; F2: 39.65 µg/mL; F3: 38.86 µg/mL), with a positive dose-response trend supported by flavonoids, alkaloids, and saponins, approaching the pure extract (IC50 42.37 µg/mL) and vitamin C (7.95 µg/mL) as a comparison.

Despite its success, limitations include the limited number of panelists (9), the absence of long-term stability testing, and the focus solely on DPPH without other antioxidant methods such as ABTS. Suggestions for further research include large-scale clinical trials, cyclic stability, and additional formulations such as serums for anti-aging applications. Practically, F3 toner has the potential to be an affordable natural cosmetic to treat xerosis and premature aging in Indonesia, supporting the local use of pumpkin to reduce dependence on synthetic materials.

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